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ORIGINAL ARTICLE

Serum Leptin levels in Rheumatoid arthritis and relationship with disease activity



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KEYWORDS

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Abstract *Aim of the work:* This study was designed to measure the serum leptin level in patients with rheumatoid arthritis, and to correlate it with clinical manifestations and disease activity.

Patients and methods: Sixty adult RA patients (58 females and 2 males) and 30 healthy subjects matching age serving as the control group, were included in this study. Assessment of disease activity was done using the DAS-28 scoring system. Calculation of body mass index (BMI) was carried out for both RA patients and controls. Measurement of the serum leptin level in RA patients and controls was done using the DRG leptin ELISA Kit.

Results: RA patients showed statistically significant higher mean serum leptin level than healthy controls (24.86 ± 26.41 versus 10.73 ± 8.19 ng/dl respectively, $P = 0.004$). In addition, serum leptin level showed a statistically significant positive correlation with body mass index ($P < 0.001$). No significant correlation was found between serum leptin level and patients' age, disease duration and disease activity. Mean serum leptin level was 25.2 ng/ml in seropositive patients and 24.5 ng/ml in seronegative patients, a finding which proved to be statistically significant when comparing the two groups ($P = 0.004$).

Conclusions: Even though serum leptin level was significantly higher in the RA patients than in the control group, no correlation was found between leptin level and clinical and laboratory parameters of disease activity. However the serum leptin level positively correlated with BMI in the RA patients.

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1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory systemic disorder that is characterized by polyarthritis with often progressive joint damage and disability, immunologic abnormalities, increased comorbidity, and premature mortality [1]. Rheumatoid arthritis is characterized by high concentrations of pro-inflammatory cytokines, such as tumour necrosis

factor- α (TNF- α), interleukin-1 (IL-1), interleukin-6 (IL-6). The excessive production of inflammatory cytokines correlates not only with joint disease activity and progression, but also with a loss of body cell mass, known as rheumatoid cachexia [2].

Leptin is a 16-kilo dalton (kDa) weight, non glycosylated cytokine-like hormone synthesized almost exclusively by white adipose tissue. Leptin and its receptors (Ob-R) share structural and functional similarities with cytokines of IL-6 family and their receptors. Leptin acts as a negative feedback signal for neurons in the central nervous system (CNS) to decrease food intake and to increase energy expenditure. Peripheral functions of leptin include regulation of endocrine function, reproduction and immunity [3].

During acute inflammation, pro-inflammatory cytokines increase circulating leptin concentration, and leptin, in turn, potentiates cytokine release from monocytes/macrophages. In addition, leptin stimulates T-cell mediated immunity and is able to induce the proliferation and differentiation of haemopoietic cells. The involvement of leptin in regulating immune function in humans is strongly sustained by the increased incidence of severe infection in subjects with genetic leptin deficiency and by the deficiencies of the immune system during starvation and malnutrition, when concentrations of leptin are low [4].

Interactions between the neuroendocrine and immune systems contribute to the pathogenesis of RA. Among the different neuroendocrine mediators, leptin plays a major role in the pathogenesis of RA. Leptin, as an adipocytokine with pleiotropic actions, may influence the inflammatory mechanisms of arthritis in humans through the induction of T helper1 responses [5]. The aim of this study was to measure the serum leptin level in patients with RA, and to correlate it with clinical manifestations and disease activity.

2. Patients and methods

This study comprised sixty adult rheumatoid arthritis patients (58 females and 2 males). They were all randomly selected from the Rheumatology and Rehabilitation outpatient clinic of El-Matardia Teaching Hospital. Patients were diagnosed according to the 1987 revised American Rheumatism Association Criteria for the classification of rheumatoid arthritis [6]. Rheumatoid factor was positive in 25 (41.7%) patients. Thirty healthy subjects of matching age to the patients served as the control group. Patients suffering from diabetes mellitus and those taking medication known to affect nutritional status or regulation of fat metabolism were excluded from this study.

We followed our committee's ethical guidelines in Cairo University and an informed consent was obtained from all subjects according to the Declaration of Helsinki; General Assembly, October 2008.

Patients were subjected to full history taking, clinical examination, laboratory and radiological investigations necessary for the diagnosis of rheumatoid arthritis. BMI was calculated for all subjects [7] and DAS-28 score was used to assess the disease activity [8].

Measurement of the serum leptin level was done using the DRG leptin ELISA kit [9]. The DRG leptin ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtitre wells are coated with

a monoclonal antibody directed towards a unique antigenic site on a leptin molecule. An aliquot of a patient sample containing endogenous leptin is incubated in the coated well with a specific rabbit anti-leptin antibody. A sandwich complex is formed. After incubation, the unbound material is washed off and an anti rabbit peroxidase conjugate is added for detection of the bound leptin. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of leptin in the patient sample.

Statistics: Statistical presentation and analysis were conducted by SPSS version 17. Numerical measures were represented as means and standard deviation. Cross tabulation was utilized to describe the relations between variables using the contingency coefficient. Unpaired Student *t*-test was used to compare between two groups in quantitative data. Mann-Whitney was used to test the statistical difference between the RA patients and controls regarding the serum leptin level. Linear correlation coefficient (*r*) was used for the detection of correlation between two quantitative variables in one group. *P* value < 0.05 was considered significant.

3. Results

The age of the sixty adult rheumatoid arthritis patients [58 females (96.7%) and 2 males (3.3%)] ranged between 25 and 55 years with a mean of 40.81 ± 9.11 years. Disease duration ranged between 6 months and 20 years with a mean of 5.48 ± 4.15 years. Thirty healthy subjects (25 females and 5 males), of matching age to the patients, served as the control group.

The clinical data of the RA patients are shown in (Table 1).

The DAS-28 score ranged between 2.8 and 8.50 with a mean of 5.37 ± 1.11 . Low disease activity < 3.6 was found in 5 (8.33%) patients, moderate disease activity ≥ 3.6 – ≤ 5.5 was found in 32 (53.33%) patients and high disease activity > 5.5 was found in 23 (38.33%) patients.

The laboratory data of the RA patients are shown in (Table 2). Rheumatoid factor results are shown in (Table 3).

A significant positive correlation was found between serum leptin level and BMI ($P < 0.001$) in RA patients. However, no significant correlation was found between mean serum leptin level and patients' age ($P = 0.72$) and disease duration ($P = 0.51$).

As regards RA activity, serum leptin level did not significantly differ in patients with low, moderate or high disease activity ($P = 0.86$). Mean serum leptin level in patients with low disease activity was 34.68 ± 48.37 compared to 22.21 ± 15.80 in patients with moderate disease activity and 26.41 ± 32.54 in patients with high disease activity (Table 4). No significant correlation was found between serum leptin level and disease activity in the RA patients (Fig. 1).

Serum leptin level in RA patients ranged between 1.10 and 122.70 ng/ml with a mean of 24.86 ± 26.41 . Serum leptin level in control subjects ranged between 0.40 and 28 ng/ml with a mean of 10.73 ± 8.19 . A statistically significant difference was found between RA patients and controls as regards the serum leptin level ($P = 0.004$). Patients with RA showed higher concentrations of leptin (almost twice the mean leptin levels) when compared to the control group (24.86 ± 26.41 versus 10.73 ± 8.19 ng/dl respectively) (Table 5). However, they did not differ significantly with respect to BMI ($P = 0.4$). Mean

Table 1 Clinical data of the RA patients.

Clinical data	Range	Mean \pm SD
Age (years)	25–55	40.81 \pm 9.11
Disease duration (years)	6 months–20 years	5.48 \pm 4.15
Morning stiffness duration (min)	60–180	87.97 \pm 29.86
Tender joint count	2–28	11.9 \pm 6.99
Swollen joint count	1–26	5.87 \pm 6.08
Body mass index (kg/m ²)	16–50	33.08 \pm 7.98

Table 2 Laboratory data of the RA patients.

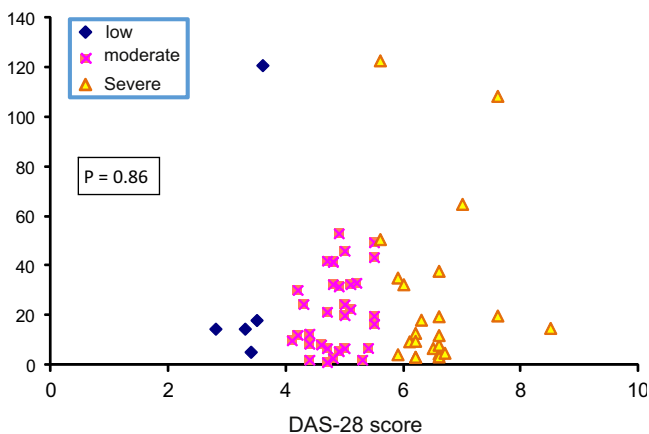
Laboratory data	Range	Mean \pm SD
ESR (mm/1st h)	15–110	44.5 \pm 18.9
Hb (gm/dl)	9.5–13.6	11.15 \pm 1.46
WBC count/mm ³	4.3–10.8	7.65 \pm 1.79
Platelet count/mm ³	205–233.3	533 \pm 730.01
Serum ALT (u/l)	9–39	19.42 \pm 9.19
Creatinine (mg/dl)	0.5–1.2	0.74 \pm 0.15
Serum leptin (ng/ml)	1.1–122.7	24.86 \pm 26.41

Table 3 Rheumatoid factor results in the RA patients.

RF	RA patients	
	Number	Percentage
Negative	25	58.3
Positive	35	41.7

Table 4 Comparison between mean serum leptin level and RA patients with low, moderate and high disease activity.

DAS-28	Serum leptin		Kruskal–Wallis test	
	Range	Mean \pm SD	χ^2	P-value
Low	5.3–120.8	34.68 \pm 48.37	0.30	0.86
Moderate	1.1–3	22.21 \pm 15.8		
High	3.2–122.7	26.41 \pm 32.54		

**Figure 1** Scatter diagram showing the correlation between serum leptin level and DAS-28 score in the RA patients with low, moderate and high disease activity.

BMI of RA patients was 33.08 \pm 7.98 and that of controls was 31.76 \pm 4.39.

A significant difference was found between RA patients with positive rheumatoid factor and those with negative rheumatoid factor as regards the serum leptin level ($P = 0.004$). Mean serum leptin level of seropositive patients was 25.27 \pm 27.16 ng/ml and that of seronegative patients was 24.57 \pm 426.26 ng/ml (Table 6).

4. Discussion

RA is a chronic inflammatory disease that affects articular as well as extra-articular structures. It is characterized by synovial hyperplasia, inflammatory cell recruitment and in its later stages cartilage and bone destruction. Several studies have demonstrated that Th1/Th2 balance plays an important role in RA, with Th1 and Th2 cytokines exerting pro-inflammatory and anti-inflammatory effects [10].

Leptin was initially described as a hormone that regulates food intake and energy balance. Later, it became apparent that leptin has an important role in regulating neuroendocrine and immune functions. Leptin and its receptors (Ob-R) share structural and functional similarities with cytokines of the interleukin-6 family and their receptors [4].

Leptin activates monocyte/macrophage cells and increases production of the pro-inflammatory cytokines (TNF- α and IL-6). Leptin also stimulates the proliferation of T-cells, directs T cell differentiation to Th1 phenotype and protects T-cells from corticosteroid-induced apoptosis [11]. Leptin also exerts anti-inflammatory activities, which are attributed to increased IL-4 production and release of IL-1 receptor antagonist [12].

Fasting patients with RA exhibit an improvement in clinical parameters of disease activity, associated with a decrease in serum leptin concentration and a shift towards Th2 cytokine production. These changes observed in RA patients and experimental models suggest, that leptin may play a role in inflammatory mechanisms of arthritis [13].

This study was designed to measure the serum leptin level in patients with RA, and to correlate it with clinical

Table 5 Comparison between RA patients and controls regarding mean serum leptin level.

Group	Serum leptin		Mann–Whitney test	
	Range	Mean \pm SD	Z	P-value
RA patients	1.10–122.70	24.86 \pm 26.41	−2.87	0.004 ^a
Controls	0.40–28.00	10.7 \pm 38.19		

^a Significant.

Table 6 Comparison between seropositive and seronegative RA patients as regards serum leptin level.

RF	Serum leptin		Mann-Whitney test	
	Range	Mean \pm SD	Z	P-value
Negative	2.10–120.80	24.57 \pm 426.2625	–2.876	0.004 ^a
Positive	1.10–122.70	25.276 \pm 27.1679		

^a Significant.

manifestations and disease activity parameters. It was conducted on 60 RA patients (58 females and 2 males) and 30 healthy subjects of matching age to the patients.

In our study, no significant correlation was found between plasma leptin level and the age of RA patients ($P = 0.72$), or disease duration ($P = 0.51$). Our results come in agreement with the results of Popa and associates [4] who found that serum leptin levels in RA patients were not related to disease duration. However, Bokarewa and colleagues [14] showed a gradual increase of leptin concentration with the duration of RA.

A significant difference was found between serum leptin level of the studied RA patients and controls. Serum leptin level in patients with RA was significantly higher than in healthy controls ($P = 0.004$). This comes in agreement with Sarraf et al. [15] who mentioned that in the process of chronic inflammatory diseases, pro-inflammatory cytokines (IL-1 and TNF- α) increase obese gene expression and leptin secretion. Also, in other studies [14,16–18], the serum leptin level was significantly higher in RA patients than controls. In a study by Otero and associates [5] considerable higher plasma levels of fat derived hormones (leptin, adiponectin and visfatin) were found in RA patients than in healthy controls. Seven and colleagues [19] found that serum and synovial fluid leptin levels were significantly higher in RA patients than the control groups.

On the contrary, Targojska-Stdpiak and colleagues [20] found that mean serum leptin concentration in their RA patients remained within normal ranges. Also, Bruun and coworkers [21] considered that long-term stimulation of adipose tissue by TNF- α or IL-1 inhibits leptin and leptin mRNA production. Harle and associates [22] found almost three times lower serum leptin concentration in a group of RA women compared to the healthy women group. Tokarczyk-Knapik and colleagues [23] suggested that chronic inflammation may lower the leptin level in plasma concentration contrary to acute inflammation. Nishiya and associates [24] found no difference in the serum leptin between RA patients and controls. In agreement, Popa and Associates [4] found that serum levels of leptin were not increased in patients with RA compared to controls. They contributed this lack of difference to a combination of factors, the most important being that a significant percentage of RA patients did not have inflammatory parameters at the time of investigation.

In this work, no significant correlation was found between the serum leptin level and disease activity in patients with low, moderate or high disease activity. This comes in agreement with the findings of other studies [14,17,18,25] that reported no statistical significant difference between the leptin level and disease activity in RA patients. Similarly, Wisolwska and associates [11] reported that no correlation was found between DAS-28 score and serum level of leptin. There was no statistical significant difference between median values of leptin level for low and high DAS-28 values.

On the contrary, Seven and colleagues [19] found that RA patients with moderate disease activity (DAS > 2.7) had significantly higher leptin levels than those with low disease activity (DAS < 2.7). Olama and colleagues [26] found that synovial/serum leptin ratio was significantly higher in RA patients with erosions than RA patients without erosions. Serum leptin level and synovial/serum leptin ratio significantly correlated with RA duration, DAS28, ESR, CRP, TNF- α and IL-6. These results indicate that local consumption of leptin in the joint cavity has a protective role against the destructive course of RA.

In addition, Yoshino and associates [27] reported that serum levels of resistin and leptin were positively associated with the CRP level in patients with rheumatoid arthritis, suggesting that these adipokines may act as pro-inflammatory cytokines in this disease. Also, Targojska-Stdpiak and colleagues [20] found a positive correlation between serum leptin concentration and DAS-28 values.

In this study, a significant positive correlation was found between serum leptin levels and BMI ($P < 0.001$) of the RA patients. Our results come in agreement with the work of other studies [11,18,24,25,28] that reported a positive correlation between BMI of RA patients and serum levels of leptin. Targojska-Stdpiak and colleagues [20] found that leptin concentration correlated positively with BMI in women with RA, but not in men.

On the contrary, Popa and coworkers [4] found that in RA patients, plasma leptin concentration did not correlate with BMI. Also, Seven and colleagues [19] reported no correlation between leptin and BMI. They suggested that regulation of leptinemia is complex and that weight is not the only major regulator.

Limitations of this study were the small number of RA male patients which did not allow us to obtain results of value to assess the effect of gender on leptin levels. Also, the number of controls was not gender matched with the patients. Correlation of radiological findings with leptin levels in the RA patients was not done; however, it would have added considerable information and would be taken into consideration in further studies.

In conclusion, circulating leptin levels did not seem to reflect disease activity in our study. Since many aspects of the biology of leptin remain unclear, we recommend further research on leptin hormone in relation to other hormonal changes in rheumatoid arthritis patients, and more studies on the influence of leptin on immunity in relation to the metabolic state aiming to reach new approaches that may focus on nutrition for the modulation of the immune response.

Conflict of interest

None.

References

- [1] Straub RH, Kalden JR. Stress of different types increases the pro-inflammatory load in rheumatoid arthritis. *Arthritis Res Ther* 2009;11:114.
- [2] Walsmith J, Abad L, Kehayias J, Roubeno VR. Tumor necrosis factor- α production is associated with less body mass in women with rheumatoid arthritis. *J Rheumatol* 2004;31:23–9.

- [3] Palmer G, Gabay C. A role for leptin in rheumatic diseases? *Ann Rheum Dis* 2003;62:913–5.
- [4] Popa C, Netea MG, Radstake TR, van Riel PL, Barrera P, van der Meer JW. Markers of inflammation are negatively correlated with serum leptin in rheumatoid arthritis. *Ann Rheum Dis* 2005;64:1195–8.
- [5] Otero M, Lago R, Gomez R, Lago F, Dieguez C, Gómez-Reino JJ, et al. Changes in fat-derived hormones plasma concentrations: adiponectin, leptin, resistin, and visfatin in rheumatoid arthritis subjects. *Ann Rheum Dis* 2006;65:1198–201.
- [6] Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:324–51.
- [7] Quinn E. BMI – What is BMI or body mass index? About.com Guide, Sports Med 2008.
- [8] Aletaha DM, Ward MM, Machloid KP, Nell VP, Stamm T, Smolen JS. Remission and active disease in rheumatoid arthritis, defining criteria for disease activity states. *Arthritis Rheum* 2005;52:2625–35.
- [9] Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 1996;334:292–5.
- [10] Busso N, So A, Chobaz-Peclat V, Morard C, Martinez-Soria E, Talabot-Ayer D, et al. Leptin signaling deficiency impairs humoral and cellular immune responses and attenuates experimental arthritis. *J Immunol* 2002;68:875–82.
- [11] Wislowska M, Rok M, Jaszczyk B, Stępień K, Cicha M. Serum leptin in rheumatoid arthritis. *Rheumatol Int* 2007;27:947–54.
- [12] Bernotiene E, Palmer G, Gabay C. The role of leptin in innate and adaptive immune responses. *Arthritis Res Ther* 2006;8:217.
- [13] Fraser DA, Thoen J, Reseland JE, Forre O, Kjeldsen-Kragh J. Decreased CD4⁺ lymphocyte activation and increased interleukin-4 production in peripheral blood of rheumatoid arthritis patients after acute starvation. *Clin Rheumatol* 1999;18:394–401.
- [14] Bokarewa M, Bokarew D, Hultgren O, Tarkowski A. Leptin consumption in the inflamed joints of patients with rheumatoid arthritis. *Ann Rheum Dis* 2003;62:952–6.
- [15] Sarraf P, Frederich RC, Turner EM, Ma G, Jaskowiak NT, Rivet 3rd DJ, et al. Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. *J Exp Med* 1997;185:171–5.
- [16] Salazar-Paramo M, Gonzalez-Ortiz M, Gonzalez-Lopez L, Sánchez-Ortiz A, Valera-González I, Martínez-Abundis E, et al. Serum leptin levels in patients with rheumatoid arthritis. *J Clin Rheumatol* 2001;7:57–9.
- [17] Gunaydin R, Kaya T, Atay A, Olmez N, Hur A, et al. Serum leptin levels in rheumatoid arthritis and relationship with disease activity. *South Med J* 2006;99:1078–83.
- [18] Allam A, Radwan A. The relationship of serum leptin levels with disease activity in Egyptian patients with rheumatoid arthritis. *Egypt Rheumatol* 2012;34:185–90.
- [19] Seven A, Guzel S, Aslan M, Hamuryudan V. Serum and synovial fluid leptin levels and markers of inflammation in rheumatoid arthritis. *Rheumatol Int* 2009;29:743–7.
- [20] Targojska-Stępnik B, Majdan M, Dryglewska M. Leptin serum levels in rheumatoid arthritis patients: relation to disease duration and activity. *Rheumatol Int* 2008;28:585–91.
- [21] Bruun JM, Pedersen SB, Kristensen K, Richelsen B. Effects of pro-inflammatory cytokines and chemokines on leptin production in human adipose tissue in vitro. *Mol Cell Endocrinol* 2002;190:91–9.
- [22] Harle P, Pongratz G, Weidler C, Büttner R, Schölmerich J, Straub RH, et al. Possible role of leptin in hypoandrogenicity in patients with systemic lupus erythematosus and rheumatoid arthritis. *Ann Rheum Dis* 2004;63:809–16.
- [23] Tokarczyk-Knapik A, Nowicki M, Wyroślak J. The relation between plasma leptin concentration and body fat mass in patients with rheumatoid arthritis. *Pol Arch Med Wewn* 2002;108:761–7.
- [24] Nishiya K, Nishiiyama M, Chang A, Shinto A, Hashimoto K. Serum leptin in patients with rheumatoid arthritis are correlated with body mass index. *Rinsho Byori* 2002;50:524–7.
- [25] Anders HJ, Rihl M, Heufelder A, Loch O, Schattenkirchner M. Leptin serum levels are not correlated with disease activity in patients with rheumatoid arthritis. *Metabolism* 1999;48:745–8.
- [26] Olama SM, Senna MK, Elarman M. Synovial/serum leptin ratio in rheumatoid arthritis: the association with activity and erosion. *Rheumatol Int* 2012;32(3):683–90.
- [27] Yoshino T, Kusunoki N, Tanaka N, Kaneko K, Kusunoki Y, Endo H, et al. Elevated serum levels of resistin, leptin and adiponectin are associated with C-reactive protein and also other clinical conditions in rheumatoid arthritis. *Intern Med* 2011;50:269–75.
- [28] Büttner R, Bollheimer LC, Zietz B, Drobnik W, Lackner K, Schmitz G, et al. Definition and characterization of relative hypo- and hyperleptinemia in a large Caucasian population. *J Endocrinol* 2002;175:745–56.